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PHARMACOLOGY AND METABOLISM OF HYBALINE A

MYRON S. WEINBERG, PhD
RICHARD E. GOLDHAMER

Food and Drug Research Laboratories, Inc.

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AEROSPACE MEDICAL RESEARCH LABORATORIES
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The experiments reported herein were conducted according to the "Principles of Laboratory Animal Care" established by the National Society for Medical Research.

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FOREWORD

This study was undertaken at the request of the Biomedical Laboratory of the Aerospace Medical Research Laboratories, Aerospace Medical Division, Wright-Patterson Air Force Base, Ohio 45433. The research was performed in accordance with Contract No. AF33(615)-2380 and in support of Project 6302, "Toxic Hazards of Propellants and Materials", and Task 630202, "Pharmacology-Biochemistry". Dr. Myron S. Weinberg was the principal investigator and Richard E. Goldhamer was co-investigator for the Food and Drug Research Laboratories, Inc., Maurice Avenue at 58th Street, Maspeth, New York, 11378. Dr. Kenneth C. Back was contract monitor for the Toxicology Branch, Toxic Hazards Division, Biomedical Laboratory, Aerospace Medical Research Laboratories. Research was initiated on 1 March 1965 and completed on 28 February 1966.

Publication of this report does not constitute Air Force approval of the report's findings or conclusions. It is published only for the exchange and stimulation of ideas.

Wayne H. McCandless
Technical Director
Biomedical Laboratory
Aerospace Medical
Research Laboratories

ABSTRACT

Toxicologic studies are described in which Hybaline A (an aluminum borohydride derivative) has been administered to rats, rabbits, cats, and dogs via the intragastric, intraperitoneal, intravascular, cutaneous, subcutaneous, and inhalation routes. All available data indicate that all effects of Hybaline A on mammalian systems can be attributed to physiochemical changes caused by energy production during hydrolysis or thermal decomposition of Hybaline A. No circulating metabolites of Hybaline A were identified. Those animals that survived the initial exposure showed no changes in any system that could be considered a pharmacodynamic action of Hybaline A.

SECTION I

INTRODUCTION

Studies were carried out to determine the pharmacodynamic activity and the metabolic fate of Hybaline A, the composition of which is:

$$53\%\ CH_3NH_2AL(BH_4)_3$$
$$47\%\ (CH_3)NH\ AL(BH_4)_3$$

The specific properties of this mixture have been described in detail (ref 1). The class of compounds of which Hybaline A is an example are all extremely sensitive to water, to other hydrogen donors, or to solvolysis causing exothermic decomposition in either the aqueous or amine system. In addition, Hybaline A itself is thermolabile, decomposing rapidly at room temperature. Thus, on standing in an inert or dry air atmosphere, the clear, colorless liquid will in a short time become a white crystalline mass. Although the results of inhalation exposure to congeners of Hybaline A have been reported (ref 2) there are no summaries of effects of parenteral, oral, or dermal exposure to these materials or of the biochemical or metabolic sequelae to this administration. Prediction of the potential industrial hazard of human exposure requires consideration of both the pharmacological and biochemical effects. This project was designed to allow for observation of general physiological reactions and particularly to evaluate the metabolic fate of any material entering the systemic circulation. Initially, the major problem involved the development of suitable techniques for the administration of the test material. Only after the design of specific chambers for its administration could the studies, which are the basis of the present report, be carried out.

SECTION II

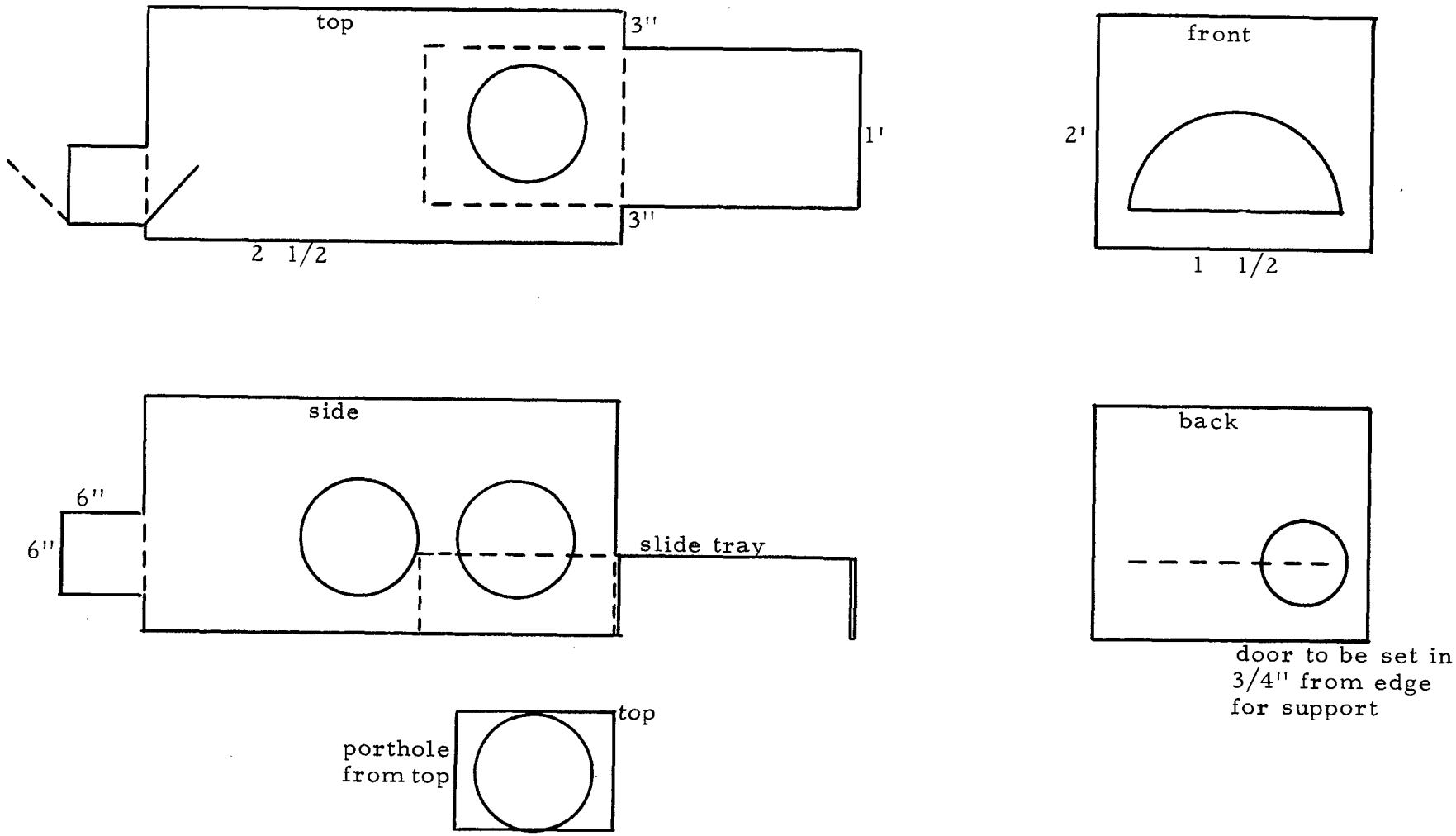
MATERIALS AND METHODS

ADMINISTRATION OF THE TEST MATERIAL

A lucite chamber was used for all dosing procedures with the exception of the inhalation studies. The animal body was extended into the chamber (fig 1) as in a plethysmograph, the head extending from a neoprene collar into the outer air. Handling of the animals and dosing equipment was accomplished by means of neoprene gloves extending into the chamber in which a dynamic helium atmosphere was maintained at the flow rate of 25 liters per minute. The gaseous effluent from the chamber was passed through a mixture of n-hexyl- and n-heptylamines to remove any residual Hybaline or other active materials. Thermolabile decomposition was prevented by maintenance of the test material in an ice-water bath until such time as it was used.

Glass microliter syringes fitted with stainless steel needles were used for all administrations.

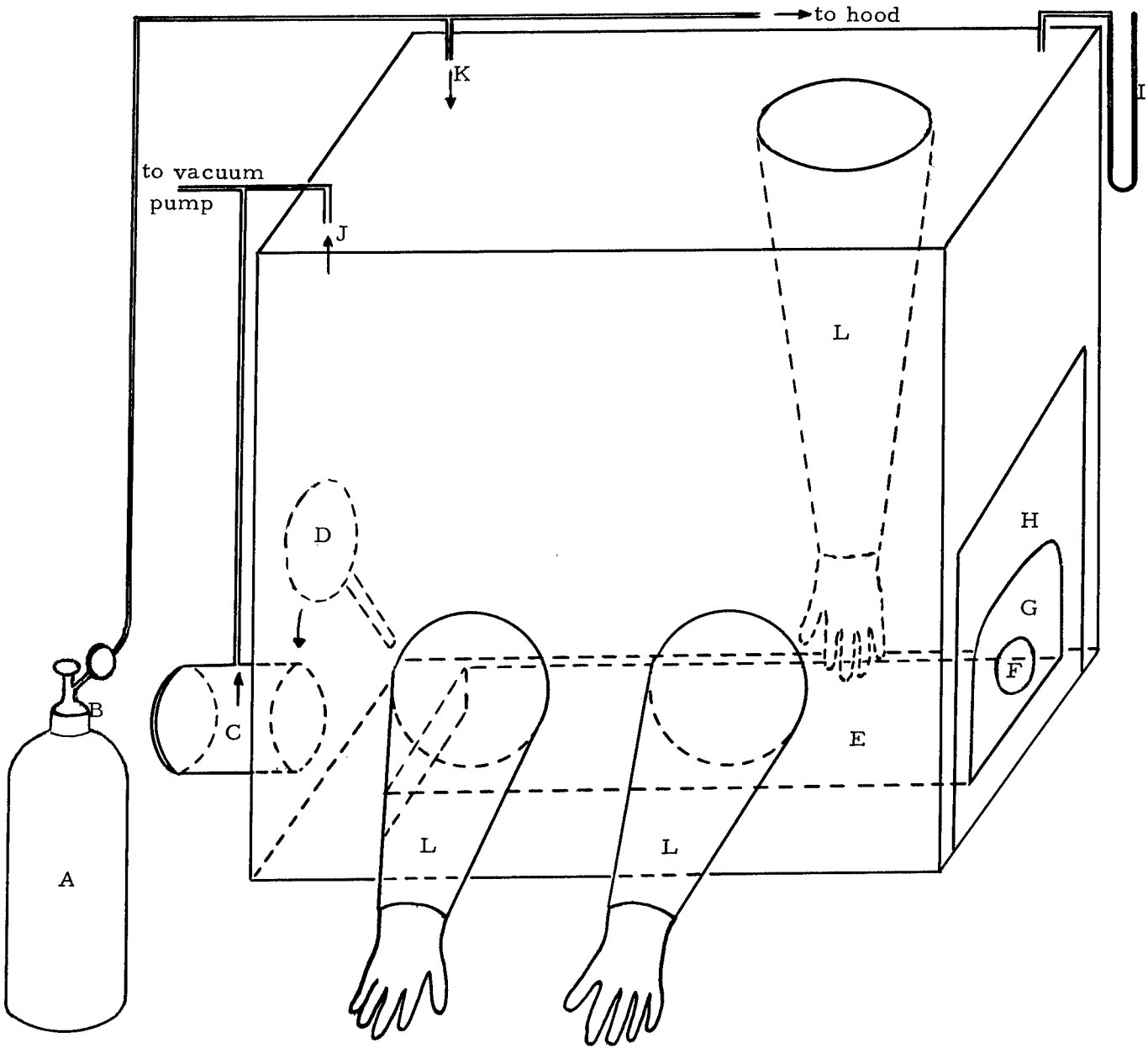
In essence, administration of the test material was made within a clear chamber in which animals could be handled by two men simultaneously, and in which the active material was maintained in an inert atmosphere.



Note: all inside dimensions 1/2" stock

Figure 1 a

Design of Helium Chamber for Animal Treatment



Letter designation

A - Compressed helium
B - Pressure valve
C - Trap door
D - Lid for trap door
E - Animal board
F - Porthole for animal's head
G - Rubber dam
H - Plexiglass cover plate
I - Manometer
J - Evacuation openings
K - Helium inlet opening
L - Plastic gloves

Figure 1 b

Helium Flow Chamber

Inhalation studies were made of the decomposition products of Hybaline A. Test animals were maintained in a vented fiberglass hood fitted with neoprene glove attachments. Using a Teflon-lined burette, liquid Hybaline A at room temperature (30 ± 5 C), was dropped into an open vessel at such a rate that rapid decomposition occurred with explosion or flame. Minimal air flow was maintained in the hood in which exposures were made to maximize the exposure to the decomposition products, with sufficient oxygen input to prevent suffocation. The effluent from the hood was passed through a Mine Safety Appliance ultra-hood filter system so that none of the decomposition products were vented into the outside air.

SECTION III

PROCEDURE

A summary of all studies involving administration of Hybaline A to rats, cats, dogs, and rabbits is shown in table 1, listing biochemical, aluminum analyses, and any other tests which were carried out.

Blood tissue aluminum levels were determined by neutron activation analysis, carried out by Nuclear Analysis Service, Union Carbide Corporation, Tuxedo, New York. Preliminary studies of these techniques indicated that less than 0.7 mg aluminum per 1000 g of test material could not be detected. For the determination of serum enzymes, the methods recommended by the Sigma Chemical Company of St. Louis, Missouri were used (ref 3). Serum protein and hemoglobin electrophoresis and other clinical laboratory determinations were carried out by the methods described by Oser (ref 4).

TABLE I

SUMMARY OF TESTS CONDUCTED

Route of Administration	No. Per Group	Dose	Survival	Necropsy	Clinical Laboratory Determinations	Aluminum Levels	Other Observations
		µl/kg	days				
Rats							
Intragastric	10	1	7	all		feces	
	"	4	"	"		urine, liver	
	"	10	"	"		kidney	
	"	40	"	"		brain	
	"	100	"	"			
	"	400	"	"			
Intraperitoneal	10	1	3	all		kidney, liver	Behavior, food
	"	5	"	"		brain, urine	consumption,
	"	10	"	"			water, urine anal-
	"	50	"	"			ysis
Intraperitoneal	20	100	7	all	GOT*, BUN, glucose	urine, feces,	
	"	50	"	"	CBC	liver, kidney,	
						brain	
Intraperitoneal	20	100	7	all	GOT levels in liver and kidney, at 0, 1, and 7 days	liver, kidney	
Subcutaneous	10	1	7	all		kidney, liver	Pain sensi-
	"	10	"	"		brain, feces,	tivity, behavior,
	"	100	"	"		urine, injec-	food consumption,
	"	400	"	"		tion sites	water intake
Subcutaneous	10	400	7	all	GOT, BUN, glucose CBC		
Intravenous	10	0.1		all			
	"	1		"			
	"	5		"			

TABLE I (continued)

Route of Administration	No. Per Group	Dose	Survival	Necropsy	Clinical Laboratory Determinations	Aluminum Levels	Other Observations
		ml/200 l**	days				
Rats							
Inhalation	10	20	21	all		lung, liver kidney	Behavior
	"	8	"	"	GOT, SLDH	lung, liver kidney	Behavior
	"	8	"	"	GOT, SLDH	lung, liver kidney	Behavior
	"	2	7	"	GOT, SLDH	lung, liver kidney	Behavior
Rabbits		mg					
Dermal	4	1000	1 hour	all	GOT, SLDH	serum, dermis, liver, kidney	
	"	100	12 hour	"	GOT, SLDH	serum, dermis liver, kidney	
Cats		µl/kg					
Intravenous	2	100					
	"	10					
	2	100					
	"	10					
Intravenous with dimethyl-formamide	1	400		all			
	"	100		"			
	"	50		"			
	"	10		"			

TABLE I (continued)

Route of Administration	No. Per Group	Dose	Survival	Necropsy	Clinical Laboratory Determinations	Aluminum Levels	Other Observations
		µl/kg	days				
Cats							
Intravenous with dimethylacetamide	1	25		all			
	"	10		"			
Intravenous with FC75 diluent	1	25		all			
	"	10		"			Emboli
	"	1		"			
Dogs							
Intra-arterial (renal artery)	3	60			serum LDH and GOT, serum and hemoglobin electrophoresis	kidney	
	1	600					
Intra-arterial (hepatic artery)	2	60			serum LDH and GOT and electrophoresis	liver	
	1	600					
Intra-arterial (carotid artery)	2	60			serum LDH and GOT and electrophoresis	brain	
	1	600					

*Glutamic oxaloacetic transaminase levels

**Allowed to decompose in a 200-liter chamber

SECTION IV

RESULTS

Initially, the material administered was that vented from the sample tank into a cooled receiving flask. The results of these studies were reported to Aerospace Medical Research Laboratories in interim reports submitted by these Laboratories. Subsequent analysis of this material led to the finding that this substance was not Hybaline A but gaseous decomposition products thereof. At that point, the experiments were initiated using the liquid samples forced from the container. Findings from the decomposition products are not included.

ACUTE INTRAVENOUS ADMINISTRATION

Acute intravenous administration of 0.1 µl or more of the test material per kg body weight to rats caused massive rupture of the infused vessel with death of the rat following exsanguination and shock. There were no signs of any effect of the material other than the traumatic blood loss from all vital tissues. Administration of dilutions of the test material with water miscible and lipophilic solvents produced essentially the same results with massive destruction of the vascular bed, shock, and mortality of all animals within minutes. Pharmacological or metabolic studies could not be carried out in rats by this route of administration.

The findings in the rats indicated that the exothermic decomposition of the test material or the energy release during hydrolysis caused the extensive trauma. Administration via a larger vessel might be possible, hence intravenous administration in cats was attempted. In these studies, three major vessels were visualized and the test material was injected directly either as received or in solution in dimethylformamide, dimethylacetamide, or FC75 solution.

Rapid infusion of 1, 10, 25, 50 or 100 µl Hybaline A into the renal, hepatic, or carotid artery of various groups of cats resulted in massive rupture of the vessel followed by extensive hemorrhage. In all instances, the cats died within minutes after the injection. In most animals the explosive reaction also caused gross injury to the nearby organs with rupture of the entire vascular bed of the organ. No metabolic studies were made in this species.

An effort to introduce the material directly into the systemic circulation continued, with the use of dogs weighing approximately 10 kg each. Preliminary studies were carried out in which a kidney, a lobe of a liver, or the prefrontal lobe of the brain could be removed and the animal maintained postoperatively for a minimum of 72 hours. These surgical procedures were carried out with a minimal level of anesthesia for visualization of the afferent and efferent blood vessels from these organs.

Following these preliminary studies, groups of dogs were treated with the pure, undiluted sample injected into the renal artery, hepatic artery, or carotid artery. While under light sodium pentobarbital anesthesia, each dog was prepared so that the particular organ of interest, i. e., the kidney, liver, or brain, could be visualized. The animal was then transferred to the treatment chamber in which the exposed organ with its afferent and efferent vasculature was within the helium atmosphere. When the anesthesia had risen to the early stages of the first plane, direct injection of Hybaline A was made into the artery at rates of 5 or 15 μl every 15 minutes for 3 hours. Samples of blood were collected from the efferent vein 5 minutes after each injection of material and at the end of the 3 hours a portion of the organ was frozen for analysis.

Tables II, III, and IV show the various studies carried out and the findings in the kidney, liver, and brain, respectively*. In addition to those dogs shown, several attempted injections had to be aborted since the equipment used for injection of the active fluid became occluded with the decomposition products and the time schedule could not be met.

Administration of 50 μl over a 15-minute period produced violent boiling within the blood vessel with destruction of the intima and precipitation of coagulated blood proteins. The dogs immediately went into shock regardless of the vessel into which the dose was introduced and required artificial maintenance. Samples of blood from efferent vessels showed increased aluminum content. Enzyme analysis indicated massive destruction of cells within the organ early in the study followed by fall in activity which may indicate depletion of enzyme stores. Serum, plasma, and hemoglobin electropherograms showed large amounts of nonmobile proteins remaining at the origin. No abnormal proteins were detected in the moving pattern. Blood glucose and urea nitrogen levels were normal.

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* Lower limits of sensitivity for each analyses are indicated on their respective tables.

TABLE II

ACUTE INTRA-ARTERIAL ADMINISTRATION OF HYBALINE A
(RENAL ARTERY IN DOGS)

Dose	Dog No, & Sex	Time	Aluminum Levels**		SGOT		Venous		Liver Aluminum Levels**
			Artery	Vein	Artery	Vein	Urea Nitrogen	Glucose	
µl		min	mg/1000 g		units/ml		mg/100 ml		mg/1000 g
50	R-1M	0	<0.7	<0.7	21	21	11	60	
		15	0.7	0.7	0	162	11	65	
		30	1.5	0.8	0	166	11	90	
		45	1.4	<0.7	0	7670	16	96	
		60	2.8	1.0	0	5250	15	89	
		75	3.7	1.9	0	5720	11	70	
		90	3.7	1.5	0	96	12	76	
		105	4.9	0.6	0	108	14	88	
		120	4.9	1.6	0	420	11	81	
		135	4.4	1.1	0	116	11	70	
		150	3.6	1.2	0	315	11	49	
		165	5.7	1.0	0	322	11	40	
		180	4.6	1.6	0	389	11	48	16.2
5*	R-2M	0	<0.7	<0.7	40	38	14	40	
		15	0.7	<0.5	0	39	14	46	
		30	1.5	<0.8	0	71	14	30	
		45	1.4	<0.8	0	161	14	60	
		60	1.8	<0.9	0	2146	14	32	
		75	1.7	<1.0	0	1740	14	31	
		90	1.7	<0.5	0	9110	14	41	
		105	1.9	<0.6	0	8610	14	42	
		120	1.9	<0.8	0	121	14	67	
		135	1.4	<0.9	0	126	14	70	
		150	1.6	<0.9	0	60	14	76	
		165	1.7	<0.9	0	192	14	80	
		180	1.6	<0.9	0	172	14	72	2.7
		720*					14		3.6

TABLE II (continued)

Dose	Dog No. & Sex	Time	Aluminum Levels**		SGOT		Venous		Liver Aluminum Levels**
			Artery	Vein	Artery	Vein	Urea Nitrogen	Glucose	
µl		min	mg/1000 g		units/ml		mg/100 ml		mg/1000 g
5	R-3M	0	<0.7	<0.7	16	17	14	40	
		15	1.7	<0.7	0	216	13	30	
		30	1.5	<0.7	0	210	14	26	
		45	1.4	<0.7	0	96	17	17	
		60	2.8	<0.7	0	420	17	60	
		75	1.6	<0.7	0	196	14	75	
		90	1.4	<0.7	0	2160	14	75	
		105	1.2	<0.7	0	2130	26	61	
		120	1.7	<0.7	0	4240	20	62	
		135	1.5	<0.7	0	7260	61	96	
		150	1.6	<0.7	0	9200	17	108	
		165	1.3	<0.7	0	8750	14	126	
		180	1.4	<0.7	0	10250	12	120	1.7
5	R-1F	0	<0.9	<0.7	42	38	11	70	
		15	0.6	<1.0	0	21	12	71	
		30	<0.5	<0.8	0	16	8	65	
		45	0.8	<0.8	0	51	17	65	
		60	1.2	<0.8	0	60	16	65	
		75	1.6	<0.8	0	1720	12	66	
		90	1.5	<0.8	0	1640	10	67	
		105	1.5	<0.8	0	1040	12	69	
		120	1.5	<0.8	0	1240	11	70	
		135	1.5	<0.8	0	6120	11	72	
		150	1.9	<0.8	0	910	11	68	
		165	1.4	<0.8	0	1090	11	64	
		180	1.4	<0.8	0	6050	11	108	2.6

* At an injection rate of 0.1 µl per minute.

** The limits of sensitivity of the neutron activation analysis for aluminum, which vary from run to run, are in part dependent on the presence of phosphorus. The lower limit is, in each case, that numerical value for which a < designation is shown.

TABLE III

ACUTE INTRA-ARTERIAL ADMINISTRATION OF HYBALINE A
(HEPATIC ARTERY IN DOGS)

Dose	Dog No. & Sex	Time	Aluminum Levels*		SGOT		Venous		Kidney Aluminum Levels*
			Artery	Vein	Artery	Vein	Urea Nitrogen	Glucose	
µl/min		min	mg/1000 g		units/ml		mg/100 ml		mg/1000 g
50	L-1F	0	<0.7	0.7	28	28	11	71	
		15	0.7	0.9	0	46	12	66	
		30	1.1	1.1	0	64	11	66	
		45	1.1	0.9	0	91	14	40	
		60	2.4	1.1	0	7110	16	33	
		75	2.8	1.2	0	6570	11	48	
		90	2.9	1.1	0	480	11	64	
		105	3.7	1.6	0	4120	11	76	
		120	3.8	1.1	0	410	11	90	
		135	3.8	1.7	0	316	19	78	
		150	4.4	1.2	0	96	12	78	
		165	4.2	1.3	0	0	11	60	
		180	4.1	1.6	0	0	12	41	6.7
5	L-2M	0	<0.7	<0.7	21	31	14	66	
		15	<0.9	<0.7	16	31	11	46	
		30	1.0	<0.7	26	26	17	54	
		45	1.1	<0.7	0	64	11	49	
		60	1.6	<0.7	0	420	12	55	
		75	1.5	<0.7	0	444	13	71	
		90	1.4	<0.7	0	3120	17	36	
		105	1.9	<0.7	0	4444	16	33	
		120	1.7	<0.7	0	6170	11	65	
		135	1.9	<0.7	0	9120	12	70	
		150	2.1	<0.7	0	7160	17	65	
		165	1.1	<0.7	0	8190	16	66	
		180	1.6	<0.7	0	7020	11	71	<0.7

TABLE III (continued)

Dose	Dog No. & Sex	Time	Aluminum Levels*		SGOT		Venous		Kidney Aluminum Levels*
			Artery	Vein	Artery	Vein	Urea Nitrogen	Glucose	
µl/min		min	mg/1000 g		units/ml		mg/100 ml		mg/1000 g
5	L-3F	0	<0.5	<0.5	26	29	17	61	
		15	1.0	<0.5	27	61	21	62	
		30	1.0	<0.5	32	76	26	66	
		45	<0.6	<0.5	61	121	21	81	
		60	<0.6	<0.5	40	61	19	66	
		75	<0.8	<0.6	0	0	22	76	
		90	<0.8	<0.8	0	0	25	74	
		105	0.7	<0.5	0	0	30	40	
		120	<0.7	<0.5	0	10	29	46	
		135	0.8	<0.5	0	0	16	66	
		150	0.9	<0.8	0	8	14	68	
		165	0.9	<0.7	0	5	10	65	
		180	0.7	<0.9	0	21	16	75	0.7

* The limits of sensitivity of the neutron activation analysis for aluminum, which vary from run to run, are in part dependent on the presence of phosphorus. The lower limit is, in each case, that numerical value for which a < designation is shown.

TABLE IV

ACUTE INTRA-ARTERIAL ADMINISTRATION OF HYBALINE A
(CAROTID ARTERY IN DOGS)

Dose	Dog No. & Sex	Time	Aluminum Levels*		SGOT		Venous		Brain Aluminum Levels*
			Artery	Vein	Artery	Vein	Urea Nitrogen	Glucose	
µl/min		min	mg/1000 g		units/ml		ml/100 ml		mg/1000 g
50	B-1M	0	<0.7	<0.5	21	29	11	68	
		15	2.6	<0.5	20	41	11	40	
		30	11.6	<0.5	42	43	11	22	
		45	2.1	<0.5	16	30	11	40	
		60	2.1	<0.5	8	30	11	60	
		75	1.7	<0.5	10	30	14	62	
		90	1.8	<0.5	12	31	11	62	
		105	1.1	<0.5	21	16	12	62	
		120	<0.7	<0.5	8	36	14	62	
		135	<0.6	<0.5	6	27	17	62	
		150	1.6	<0.5	14	30	21	62	
		165	1.2	<0.5	8	26	11	62	
		180	2.2	<0.5	5	18	11	62	<0.7
5	B-2M	0	<0.5	<0.7	39	46	26	68	
		15	<0.9	<0.7	46	40	19	70	
		30	1.1	<0.7	40	61	20	72	
		45	1.1	<0.7	61	39	21	70	
		60	0.9	<0.7	17	38	19	66	
		75	<0.6	<0.7	27	60	27	66	
		90	<0.6	<0.7	6	56	19	72	
		105	<0.8	<0.7	6	42	16	66	
		120	0.7	<0.7	6	40	17	68	
		135	0.7	<0.7	0	16	16	46	
		150	0.9	<0.7	0	36	21	42	
		165	0.9	<0.7	6	26	26	60	
		180	0.9	<0.7	0	46	30	72	<0.9

TABLE IV (continued)

Dose	Dog No. & Sex	Time	Aluminum Levels*		SGOT		Venous		Brain
			Artery	Vein	Artery	Vein	Urea Nitrogen	Glucose	Aluminum Levels*
µl/min		min	mg/1000 g		units/ml		mg/100 ml		mg/1000 g
5	B-3F	0	<0.7	<0.7	29	29			
		15	<0.7	<0.7	20	61			
		30	1.0	<0.7	29	70			
		45	1.0	<0.7	0	62			
		60	<0.7	<0.7	0	128			
		75	<0.7	<0.7	0	127			
		90	0.8	<0.7	0	256			
		105	2.7	<0.7	0	280			
		120	2.3	<0.7	0	260			
		135	2.4	<0.7	0	120			
		150	6.2	<0.7	0	62			
		165	6.8	<0.7	0	60			
		180	6.7	<0.7	0	39			<0.9

* The limits of sensitivity of the neutron activation analysis for aluminum, which vary from run to run, are in part dependent on the presence of phosphorus. The lower limit is, in each case, that numerical value for which a < designation is shown.

The dogs treated with 5 μl Hybaline A survived the 3-hour administration period although significant physical changes were seen in the character of the vasculature and in the blood during the injection of these minor amounts of material. Despite the fact that the injection rate was approximately 0. 1 μl per minute, the quantity involved was sufficient to cause localized hemagglutination and protein denaturation with the destruction of the renal intima. No aluminum was found in the efferent blood. Serum transaminase (SGOT) activity increased more slowly than with the larger dose but reached higher levels. Although blood urea and glucose levels varied widely, most values were within normal limits. The variations probably reflected an anesthetic effect rather than Hybaline A toxicity.

Throughout the injection period, there were signs of emboli formation and shock, and the condition of the lungs at necropsy indicated cyanosis in all dogs. The 5 μl per 15-minute treated-dogs died within 12 hours. Findings at necropsy indicated that mortality was neither the result of the operative procedure, nor of any activity of the test material, but due to the extensive anoxia, vascular and thromboembolic alteration with consequent vascular and respiratory collapse.

In summation, then, the data from the intravascular studies in rats, cats, and dogs show that mortality and all other changes reflect a physical effect of the material. No pharmacological or metabolic studies could be carried out with Hybaline A administered intravenously.

ACUTE INTRAGASTIC ADMINISTRATION

Groups of 10 mature rats (5 rats of each sex) of the FDRL strain ranging in weight from 200 to 300 g were given 1, 4, 10, 40, 100, or 400 μl Hybaline A per kg body weight. The results of these tests are shown in table V.

Those rats that died did so within 5 minutes of administration regardless of the dosage. Necropsy findings in these animals indicated that the massive evolution of energy resulting from contact of Hybaline A with gastric juice resulted in complete destruction of the viscera with bleeding into the abdominal and thoracic cavities. Within the pools of blood were collections of white crystalline masses similar to those seen upon mixing Hybaline A with the n-heptyl- and n-hexylamines in the effluent trap referred to earlier. These materials are evidently products of the solvolysis reaction of this active material with hydrogen donors. In addition to the destructive effects of direct administration of Hybaline A, portions of uninjured organs showed heat effects.

TABLE V

ACUTE INTRAGASTRIC ADMINISTRATION OF HYBALINE A IN RATS

Dose	No. Rats *	Food Consumption	Mortality	Tissue Aluminum Levels **+	SGOT	Glucose	Remarks
µl/kg		g/day	per cent	mg/1000 g	units/ml	mg/100 ml	
Control	10	16.1	0	<0.7(7)***	235(7)	44(7)	
1	10	6.2	30 in 5 minutes	<0.7(1) <0.7(7)	210(1) 261(7)	44(1) 48(7)	no detectable aluminum in blood, feces or urine
	10	7.6	50 in 5 minutes	<0.7(7)	236(7)	44(7)	no detectable aluminum in blood, feces or urine
	10		20 in 5 minutes				hemoglobin and plasma protein electropherograms normal (1, 7)
4	10	3.6	40 in 3 minutes	<0.7(1) <0.7(7)	237(1) 248(7)	48(1) 60(7)	no detectable aluminum in blood, urine or feces
10	10		100 in 3 minutes				
40	10		100 in 3 minutes				
100	10		100, immediately				
400	10		100, immediately				

*Equal numbers of males and females.

**Brain, liver, and kidney tissues from 2 rats were analyzed at each time period

***Parenthetical figures indicate the day on which the analyses were made.

+The limits of sensitivity of the neutron activation analysis for aluminum, which vary from run to run, are in part dependent on the presence of phosphorus. The lower limit is, in each case, that numerical value for which a <designation is shown.

TABLE VI

INTRAPERITONEAL ADMINISTRATION OF HYBALINE A IN RATS

Dose	No. Rats*	Average Food Consumption	Mortality days			Tissue Aluminum Levels**+	Average GOT Value***		Remarks
			<1	1-4	5-7		Liver	Kidney	
µl/kg		g/day	per cent			mg/1000 g	units/g		
Control	10	16.1	0	0	0	<0.7 (7)****	4220-9760(7)	1050-3720(7)	
1	10		0	100		<0.7 (4)			
5	10	0.9	0	100		<0.7 (4)			
10	10		0	0	50	<0.7 (4)			
25	10		20	50	30	<0.7 (4)			
50	10		100						
	10		50	50					
100	10		100						no detectable aluminum levels in blood, urine or feces
	20	2.6	40	20	20		7160-9716(1) 5160-8060(4) 7070-10600(7)	2120-5620(1) 1650-6420(4) 1350-4710(7)	no detectable aluminum level in liver and kidney
	10		60	40					
400	10		100						

*Equal numbers of rats per sex.

**Average liver, kidney and brain analyzed where indicated.

***Representative untreated animals were sacrificed and tissues analyzed to provide control data.

****Parenthetical figures indicate the day on which the analyses were made.

+The limits of sensitivity of the neutron activation analysis for aluminum, which vary from run to run, are in part dependent on the presence of phosphorus. The lower limit is, in each case, that numerical value for which a < designation is shown.

TABLE VII

ACUTE SUBCUTANEOUS ADMINISTRATION OF HYBALINE A IN RATS

Dose	No. Rats *	Average Food Consumption **	Mortality days <1	1-4	5-7	Tissue Aluminum Levels***+	Blood GOT	Glucose	Urea Nitrogen
µl/kg		g/day	per cent			mg/1000 g	units/ml	mg/100 ml	
Control	10	16.1	0	0	0	<0.7	270(7)****	48(7)	11(7)
1	10	15.8	0	0	0	<0.7	291(1)	46(1)	12(1)
10	10	16.6	0	0	0	<0.7	242(1)	44(1)	11(1)
100	10	12.2	10	0	10	<0.7	317(1)S	49(1)	11(1)
						<0.7	282(7)	46(1)	12(7)
400	10	10.2S*****	10	30	0	<0.7	392(1)S	50(1)	14(1)
							216(7)	50(7)	11(7)
	10	10.6S	30	10	30	<0.7	570(1)S	52(1)	11(1)
							300(7)	48(7)	12(7)
	10		40	20	20				
1000	10		100						

* Equal numbers of males and females.

** For 7 days.

*** Average liver and kidney analyzed on 7th day.

**** Parenthetical values represent postdose day on which determination was made.

***** S = significantly ($p<0.05$ by the "t" test) different from controls.

+ The limits of sensitivity of the neutron activation analysis for aluminum, which vary from run to run, are in part dependent on the presence of phosphorus. The lower limit is, in each case, that numerical value for which a < designation is shown.

Behavior in survivors was within normal limits although food intake was markedly depressed. This was explained by the postmortem findings 7 days after dosage, which included ulceration of the gastric and esophageal mucosa and in 2 rats of the 4 μl per kg group, penetrating lesions through the entire gastric wall involving both mucosal and serosal surfaces. The characteristic white crystalline materials were not found at necropsy.

Analyses of kidney, liver, and brain of representative animals in all groups revealed no detectable aluminum. Daily blood, urine, and fecal collections from survivors also failed to show any traces of this material. Survivors received only about 1.2 μl of Hybaline A so that its distribution in 3 g of feces would probably not be detectable. These data do not necessarily indicate that there was no absorption of the aluminum-containing moiety, but rather that none of its metabolic products could be detected at tolerated levels.

With the finding that doses which did not cause traumatic death caused no apparent behavioral or metabolic effects, this phase of the study was concluded.

ACUTE INTRAPERITONEAL ADMINISTRATION

The effort to find a route of administration which would permit evaluation of pharmacological effects of Hybaline A was extended to include the intraperitoneal route following the procedures described above.

The results of these experiments are outlined in table VI. As before, wide variations in mortality were observed among the groups of rats. No true LD_{50} dose could be calculated from these data. Such inconsistencies are associated with deaths caused by physical means, as are seen on intravenous administration of insoluble materials which may cause pulmonary or coronary thromboses, shock, or other nonpharmacodynamic reactions. In this instance, the exact placement of the injection needle within the peritoneum affected the response as did the rate of introduction of the test material into this cavity.

Despite these variations a large number of animals survived fairly high doses of Hybaline A. There were no signs in any of the survivors of effects of the test material on normal metabolism or on the activity of metal linked enzymes such as transaminase.

Marked reduction in voluntary activity and food intake was noted in the surviving groups. At necropsy of these animals, masses of white crystalline material considered to be a solvolysis product of Hybaline A were seen and evidently contributed to discomfiture of the animal manifested by reduced motility and appetite. In addition, necropsy revealed extensive trauma to the serosal surface of the gastrointestinal tract and to the outer surface of visceral organs near the site of injection.

All findings from this phase of the study indicated that effects seen were due to physicochemical reactions of the test material with the body fluids and that no pharmacodynamic or metabolic effects of the test material could be detected at tolerated levels.

ACUTE SUBCUTANEOUS ADMINISTRATION

Larger doses of Hybaline A were tolerated by this route than by any other. As indicated by the summary of data in table VII, as much as 60 percent of an adult group of rats survived 7 days after administration of 400 μl per kg. As in all other instances, deaths appeared to be due to traumatic injury at the site of injection. There were no discernible levels of aluminum in urine, blood, feces, kidney, brain, or liver of animals immediately after injection or 7 days after dosage. The specific difference between subcutaneously treated and parenterally or orally treated animals was in the minimal increases in circulating glutamic-oxaloacetic transaminase activity immediately after exposure. The increased enzyme activity may have resulted from cutaneous tissue destroyed during introduction of the test material. Only normal levels of this enzyme could be found 7 days postadministration. Other criteria of response used in these studies were normal.

Behavior was generally normal although animals in the 400 μl per kg group showed minimal voluntary activity during the observation period. Food intake was reduced in this group although the level was sufficient to support survival.

Necropsy findings were minimal and confined to the site of injection where large crystals of the now-familiar white decomposition product of Hybaline A were found. In those animals that died immediately or within 3 days after injection there were more extensive signs of trauma, frequently with complete sloughing of all dermal tissue around the area of treatment, and rarely with massive hemorrhage, the probable cause of death. In rats that survived 7 days after treatment the crystals were enclosed in rudimentary fibrous tissue.

The findings in these studies indicated lack of chemical toxicity of Hybaline A. The highest tolerated doses of the test material were administered subcutaneously. Indications were that only minimal amounts of the decomposition products were absorbed while the remainder acted in a manner similar to surgically-inserted talc, causing a foreign body reaction. We assumed that the crystals would eventually be encapsulated and might permanently remain as a sealed area within the dermis.

Thus, previous findings were confirmed in that the reactions to Hybaline A administered were characteristic of the physicochemical responses to an insoluble material, rather than pharmacodynamic in nature.

ACUTE DERMAL ADMINISTRATION

Groups of four rabbits maintained in the chamber under helium atmosphere were treated by topical administration of 0.1 or 1.0 μl/kg Hybaline A. After 3 hours of contact, the animals were removed. Samples of blood were drawn from each of the rabbits midway during the contact period, and at the time of sacrifice, i.e., 30 minutes after exposure to air.

The skin of the rabbits showed no changes during treatment. However, immediately on exposure to air the hair caught fire and the skin became severly irritated and charred. The findings in the studies are shown in table VIII. There were no changes in the oxaloacetic transaminase or aluminum content of the serum of rabbits during exposure in helium atmosphere. However, increases were noted in all serum enzyme levels in blood drawn from the rabbits 30 minutes after exposure to air. All blood samples showed extensive hemolysis.

Dehydration with laking of erythrocytes is often seen in test animals following heat destruction of the epidermal barrier.

For humane reasons the rabbits were sacrificed immediately after the 30-minute postdosage sampling. Findings at necropsy showed effects of hemolysis in the heart and lung tissue and occult blood was found in the urine.

No biological significance was attributed to these findings in the topically-treated rabbits and Hybaline A can be assumed to cause pharmacologic or metabolic alterations, but all effects were due to the physical activity of the test material.

TABLE VIII

ACUTE DERMAL ADMINISTRATION OF HYBALINE A IN RABBITS

Dose	Rabbit No. & Sex	Time	SGOT	Serum Aluminum	Glucose	BUN	Plasma Hemoglobin
µl/kg		minutes	units/ml	mg/liter		mg/100 ml	
0.1	D-1F	0	16	<0.7**	61	14	0
		90	21	<0.5	60	16	0
		150*	117	<0.5	20	15	600
	D-2F	0	26	<0.5	65	12	0
		90	24	<0.5	65	14	0
		150*	190	<0.5	44	13	750
	D-3M	0	39	<0.7	76	15	0
		90	36	<0.7	81	14	0
		150*	316	<0.7	26	17	420
	D-4M	0	16	<0.7	67	15	0
		90	18	<0.7	67	16	0
		150*	176	<0.7	41	15	960
1.0	D-5F	0	35	<0.5	90	14	0
		90	41	<0.5	85	16	0
		150*	716	<0.6	20	18	360
	D-6F	0	29	<0.7	61	21	0
		90	16	<0.7	61	17	0
		150*	82	<0.7	46	16	650
	D-7M	0	36	<0.7	76	15	0
		90	35	<0.7	66	26	0
		150*	71	<0.7	10	16	400
	D-8M	0	26	<0.7	59	17	0
		90	21	<0.5	65	18	0
		150*	410	<0.5	40	11	1060

* 30 minutes after exposure to air

** The limits of sensitivity of the neutron activation analysis for aluminum, which vary from run to run, are in part dependent on the presence of phosphorus. The lower limit is, in each case, that numerical value for which a < designation is shown.

INHALATION STUDIES IN RATS

Using a closed inhalation chamber of 200-liter capacity, groups of 10 rats were exposed to the decomposition products of Hybaline A in dry air as follows:

Group	Treatment
I	A single spray of 20 ml Hybaline A into the dry air, the decomposition products being maintained in the chamber for one hour
II	8 ml Hybaline A sprayed into the chamber over a one-hour period (i.e., at the rate of approximately 2 μl per second) under dynamic dry air flow of 25 liters per minute
III	2 ml Hybaline A sprayed into the chamber over a one-hour period (0.5 μl per second) with a diluting air flow rate of 25 liters per minute

The findings are summarized in table IX.

In Group I all animals died within 15 minutes of exposure. There were signs of extensive traumatic injury throughout the lung and from the necropsy findings death appeared to be due to heat exposure rather than to any specific effect of inhalation of Hybaline A or its decomposition products. The postmortem findings were very similar to those seen in animals exposed to large amounts of heat from open flame or other types of exothermic reactions.

The rats in Group II died either during the exposure or during the ensuing 24-hour period. Like the animals in Group I, they showed signs of extensive heat injury in the cardiopulmonary tissues.

TABLE IX

INHALATION EXPOSURE TO THE DECOMPOSITION PRODUCTS OF HYBALINE A IN RATS

Group	No. of Rats	Mortality	Serum		Blood Aluminum	Electropherograms		Alveolar Aluminum Levels	Remarks
			GOT	LDH		Hemoglobin	Serum Protein		
		percent	units/ml		mg/l +			mg/1000 g +	
I	10	100*				N	N	9.2	
II	10	100*	316	2620	<0.7	N	N	0.9	
III	10	0*	268	5610	<0.7	N	N		isoenzyme pattern shows increase in LDH_5
		60**	281	1016	<0.7	N	N	<0.7	
IV	20	Sac	292	6120	<0.7	N	N	2.6	
V	20	Sac	326	4800	<0.7	N	N	3.1	
VI	20	Sac	292	516	<0.7	N	N	<0.7	

*Within 24 hours of exposure

**Within 2 days of exposure

Sac = sacrificed 4 hours after exposure

N = normal

+The limits of sensitivity of the neutron activation analysis for aluminum, which vary from run to run, are in part dependent on the presence of phosphorus. The lower limit is, in each case, that numerical value for which a < designation is shown.

In addition, there were indications of inhalation of large solid particles followed by pulmonary hemorrhage and respiratory death. Examination of the lungs revealed characteristic white crystalline material in the alveoli. Samples of blood taken from these rats contained no aluminum, although elevated serum lactic dehydrogenase activity was noted.

Exposure to 2 ml Hybaline A over a 1-hour period caused no immediate mortality (Group III). Six of the ten animals in group III died during the first 7 days after exposure. All animals showed signs of extensive pulmonary hemorrhage and congestion, indicating that death was probably due to injury or destruction of lung tissue. There were no significant levels of aluminum (<0. 7 ppm) or changes in SGOT levels in the blood of any of these animals. Serum lactic dehydrogenase activity was increased in all of these animals as well as in survivors sacrificed at the conclusion of the 7-day observation period. The increase was due to elevated LDH_5 believed to be found in pulmonary tissue. This finding appeared to indicate that there was extensive pulmonary destruction with exposure to Hybaline A. Examination of alveolar tissue from these animals revealed the presence of white crystalline material which was believed to be the abrasive substance leading to the destruction of the alveolar wall.

Group	Treatment
IV	2 ml Hybaline A introduced into the chamber in a unit amount and animals maintained in contact for one hour
V	2 ml Hybaline A introduced into the chamber at the rate of 33 µl per minute so that one hour was required for complete exposure
VI	Not exposed to test material

All 60 animals were sacrificed after 4 hours and examined postmortem.

All animals showed signs of extensive pulmonary irritation excluding those in Group VI. Both groups exposed to Hybaline A (Groups IV and V) showed massive pulmonary hemorrhages. The lungs of Group IV were very friable. Examination of these tissues revealed white crystalline particles in Group V, but not in Group IV. The latter showed large amounts of amorphous white to gray material in the trachea and upper airways but no apparent penetration to the actual alveolar surface. The total aluminum content of the lungs was markedly increased while that of the serum was below detectable levels.

There were no changes in serum protein, hemoglobin electropherograms, nor enzyme activity of lung tissue per gram when checked for glutamic oxaloacetic transaminase, glutamic pyruvic transaminase, or lactic dehydrogenase activities. Serum glutamic oxaloacetic and glutamic pyruvic transaminase levels remained approximately within normal limits while the lactic dehydrogenase level was increased ten-fold, apparently due to increased levels of LDH_5.

Penetration to the alveoli following inhalation exposure of Hybaline A resulted in hydrolytic reactions with localized trauma.

SECTION V

DISCUSSION

During the course of these studies, Hybaline A has been administered intragastrically, topically, parenterally, and by inhalation. In all instances mortality was associated with local trauma following exothermic decomposition or solvolysis of the aluminum borohydride complex. Generally, there were residual white needle-like crystals at the site of the trauma very similar in microscopic appearance to those obtained on passage of Hybaline A through C_6- or C_7-amines. The traumatic effects of administration of this mixture were assumed to be due to interaction of hydrogen donors with Hybaline A.

Subcutaneous administration appeared to be the most acceptable route by which the material could be given systemically. There were no effects of administration of as much as 400 µl of the test material in some animals indicating that the complex had no pharmacological activity per se nor did it interfere with normal metabolic patterns in any way. Pockets of the crystals were found at the site of injection partially encapsulated in rudimentary fibrous tissue. This reaction is very similar to that seen on intraperitoneal or subcutaneous introduction of relatively insoluble inorganic materials such as talc or boric acid.

Administration by inhalation involved exposure of the test animals to decomposition products of the thermolabile material. Where the decomposition itself did not cause trauma and death, the animals showed no systemic effects.

All changes seen were essentially the results of administration of an insoluble, pharmacologically-inert, chemically-reactive material which produced only local physicochemical changes, in most instances incompatible with survival. Where the changes could be tolerated, there was no interference in normal activity.

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13. ABSTRACT

Toxicologic studies are described in which Hybaline A (an aluminum borohydride derivative) has been administered to rats, rabbits, cats, and dogs via the intragastric, intraperitoneal, intravascular, cutaneous, subcutaneous, and inhalation routes. All available data indicate that all effects of Hybaline A on mammalian systems can be attributed to physiochemical changes caused by energy production during hydrolysis or thermal decomposition of Hybaline A. No circulating metabolites of Hybaline A were identified. Those animals that survived the initial exposure showed no changes in any system that can be considered a pharmacodynamic action of Hybaline A.

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